Hyperbranched PEI with Various Oligosaccharide Architectures

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Supporting Information

Hyperbranched PEI with various oligosaccharide architectures: Synthesis, characterization, ATP complexation and cellular uptake properties

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Calculation of the degree of functionalization (DF) and total degree of functionalization (TDF) of modified PEI based on PEI-II from elemental analysis

Example for **2-Mal**:

Elemental analysis: C = 44.42 %, N = 3.94 %, H = 7.19

DP = 84, a = number of coupled maltose
$M_{Polymer}$ = (C_2H_5N) x 84 + a x ($C_{12}H_{23}O_{10}$)
Nitrogen content: N = 84 x 14 / $M_{Polymer}$
Carbon content: C = (2 x 12 x 84 + a x 12 x 12) / $M_{Polymer}$

N/C ratio is 3.94 / 44.42, a = 77.7

The degree of functionalization (**DF**) based on the conversion of twice T units and one L unit: 77.7 / (2 x 27.4 + 30.8) = 91 %

The degree of total functionalisation (**TDF**) based on the conversion of all branching units in the PEI derivative (twice T units, one L unit and one D unit): 77.7 / (2 x 27.4 + 30.8 + 25.8) = 70 %

Calculation of the degree of branching units (T, L or D units) of modified PEI based on PEI-II from elemental analysis

Example for **2-Mal**:

Number of coupled maltose units (a) on PEI-II is 77.7 received from the calculation of DF. **PEI-II** possesses 27.4 T units, 30.8 L units and 25.8 D units (Table 3) at which twice conversion of T units is possible finally to result into D units.
The calculation bases on the assumption that at first the T units are converted into L units and then the L units can be converted into D units. Therefore, 27.4 T units are converted into 27.4 L units. From all L units (Σ 58.2) 50.3 units are also converted into 50.3 D units. The final calculation gave that 7.9 L units (9.4 %) and 76.1 D units (90.6 %) are present in 2-Mal-I.

Calculation of the molecular weight (M_n) for the PEI derivative used in the ITC study

Need of DP and M_n of the parent PEI (**PEI-II** and **PEI-II**) which is presented in Table 3.
Need of number of chemically coupled oligosaccharide received by calculation of the degree of functionalization (DF) from elemental analysis.

Example for **4-Mal**:

DP = 84, M_n = 3600 g/mol, a = number of coupled maltose
$M_{Polymer}$ = (C_2H_5N) x 84 + a x ($C_{12}H_{23}O_{10}$)
Nitrogen content: N = 84 x 14 / $M_{Polymer}$
Carbon content: C = (2 x 12 x 84 + a x 12 x 12) / $M_{Polymer}$

N/C ratio is 8.09 / 44.53, a = 31

Then, determination of M_n of chemically coupled maltose unit on PEI-core with a = 31. This means the calculation of 31 x ($C_{12}H_{23}O_{10}$) followed by the addition of $M_{n,PEI}$ and $M_{maltose}$. Thus, the sum of M_n is 13800 g/mol for **4-Mal**

Figure Caption for Supporting Information

Figure 1-SI ^{1}H spectra of **1-Mal-III** and **5-Mal-III** obtained from substrate ratio **PEI-I**/**Mal-III** 1 : 5 and **PEI-III**/**Mal-III** 1: 2, respectively.

Figure 2-SI ^{1}H NMR spectrum of **2-Lac** obtained from substrate ratio **PEI-II**/**Lac** 1 : 5.

Figure 3-SI ^{1}H spectra of **4-Mal** and **6-Mal** obtained from substrate ratio **PEI-II**/**Mal** 1 : 0.5 and 1 : 0.2, respectively.

Figure 4-SI ^{1}H NMR spectrum of **6-Mal-VII** based on the substrate ratio **PEI-II**/**Mal-VII** 1 : 0.5 (R = reductively coupled maltoheptaose unit).

Figure 5-SI ^{13}C NMR spectra of (A) **1-Mal-III** based on the substrate ratio **PEI-I**/**Mal-III** 1 : 5 and (B) **3-Mal-III** based on the educt ratio **PEI-III**/**Mal-III** 1 : 2.

Figure 6-SI ^{13}C NMR spectra of **2-Mal** obtained from substrate ratio **PEI-II**/**Mal** 1 : 2 and 1 : 10, respectively.

Figure 7-SI ^{13}C NMR spectrum of **2-Lac** and **4-Lac** based on the substrate ratio **PEI-II**/**Lac** 1 : 5 and 1 : 0.4, respectively.

Figure 8-SI ^{13}C NMR spectra of **2-Mal-III** based on the substrate ratio **PEI-II/Mal-III** 1 : 5.

Figure 9-SI. ^{13}C NMR spectra of (A) **4-Mal-III** based on the substrate ratio **PEI-II**/**Mal-III** 1 : 0.5

Figure 10-SI ^{13}C NMR spectrum of **6-Mal-VII** based on the educt ratio **PEI-II**/**Mal-VII** 1 : 0.5 (R = reductively coupled maltoheptaose unit).

Figure 11-SI ATR-IR spectrum of **PEI-II**.

Figure 12-SI ATR-IR spectrum of **2-Glc** with structure **A**.

Figure 13-SI ATR-IR spectrum of **2-Mal** with structure **A**.

Figure 14-SI ATR-IR spectrum of **5-Mal-III** with structure **B**.

Figure 15-SI ATR-IR spectrum of **4-Mal** with structure **B**.

Figure 16-SI ATR-IR spectrum of **2-Lac** with structure **A**.

Figure 17-SI Binding of ATP to **PEI-III** and **3-Mal-III** and **7-Mal-III** which possess **PEI-III** as core molecule.

Figure 18-SI -fold increase in nucleotide uptake upon complexation (HepG2 cells): procedure as mentioned for Figure 8.

Table 1-SI Influence of the substrate ratio **PEI-II** : oligosaccharide (OS) and **PEI-III** : OS on the degree of functionalization (DF), total degree of functionalization (TDF) of modified PEI and the determination of the degree of T, L and D units obtained from elemental analysis.

Table 2-SI Comparison of ^{13}C chemical shifts of D, L and T units for **PEI-II** and **PEI-III** and (oligo-)saccharide-modified PEI based on modified **PEI-II** and **PEI-III** in D_2O.

Table 3-SI ^{13}C signal assignment for PEI-bonded glucose (**Glc**), maltose (**Mal**) and maltotriose (**Mal-III**)

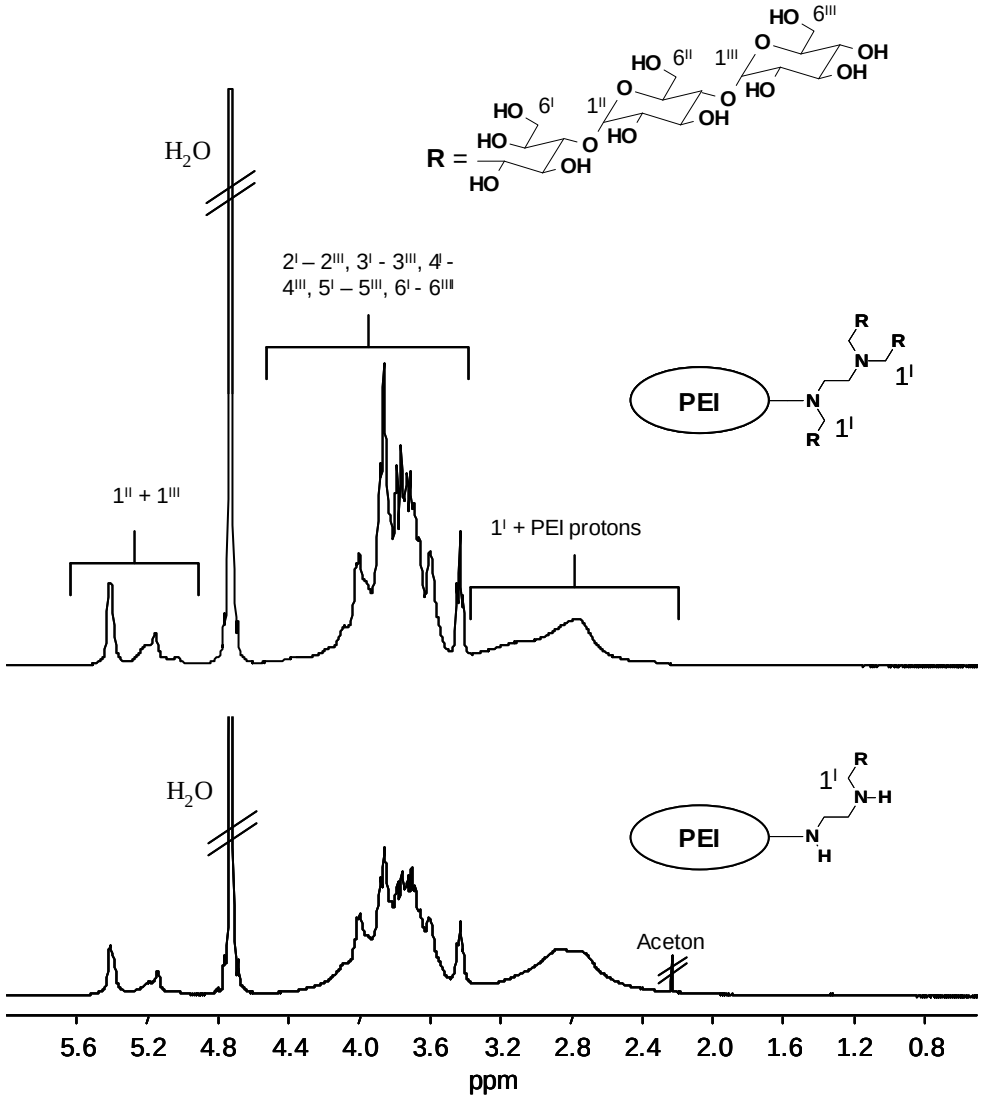

Figure 1-SI ^{1}H spectra of **1-Mal-III** with structure **A** (top) and **3-Mal-III** with structure **B** (bottom) obtained from educt ratio **PEI-I**/**Mal-III** 1 : 5 and 1: 2, respectively.

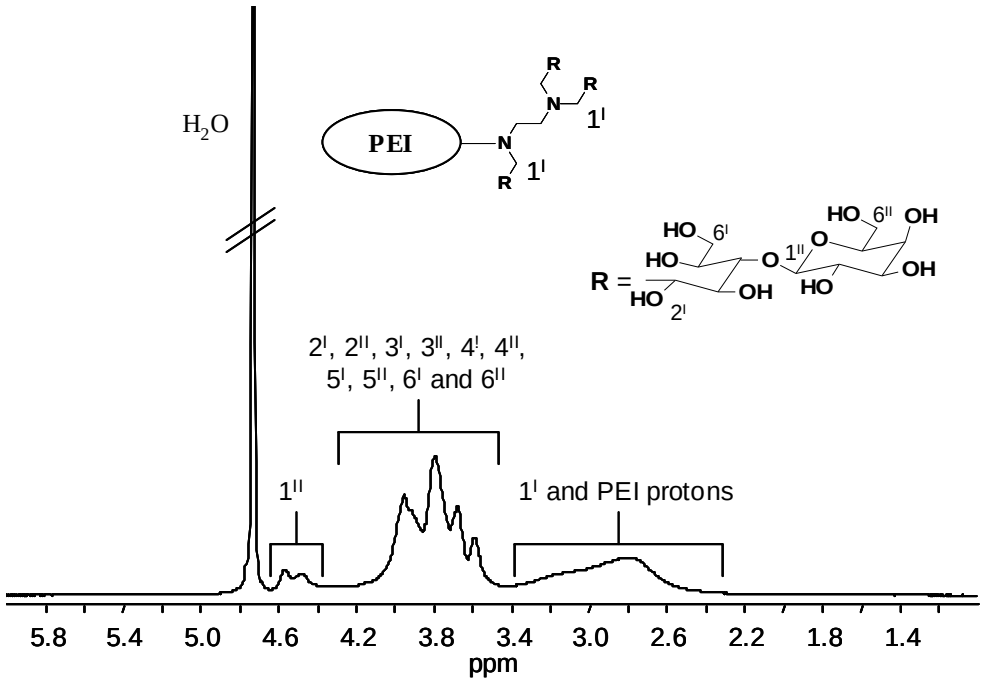

Figure 2-SI ^{1}H NMR spectrum of **2-Lac** with structure **A** obtained from educt ratio **PEI-II/Lac** 1 : 5.

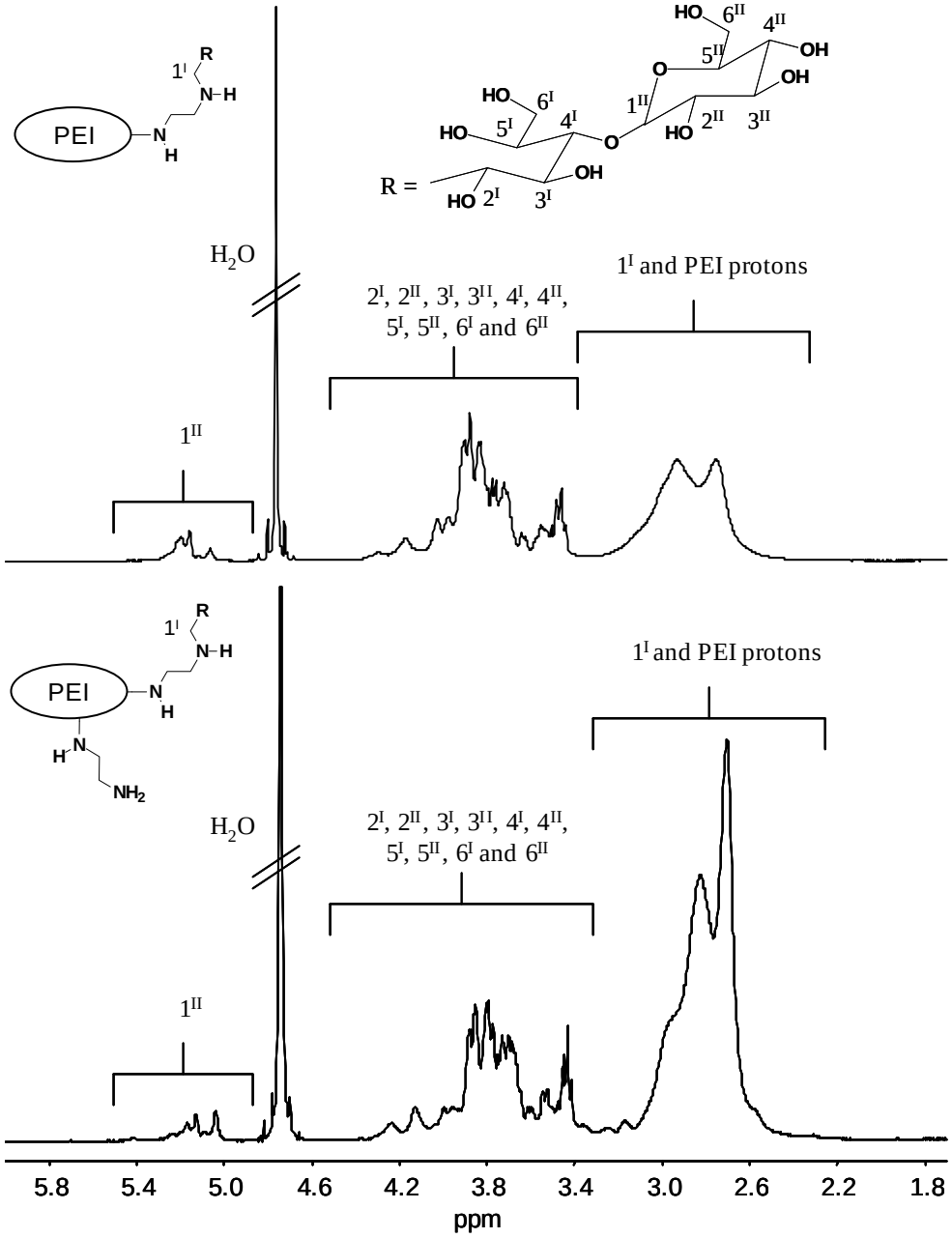

Figure 3-SI ^{1}H spectra of **4-Mal** with structure **B** (top) and **6-Mal** with structure **C** (bottom) obtained from educt ratio **PEI-II**/**Mal** 1 : 0.5 and 1 : 0.2, respectively.

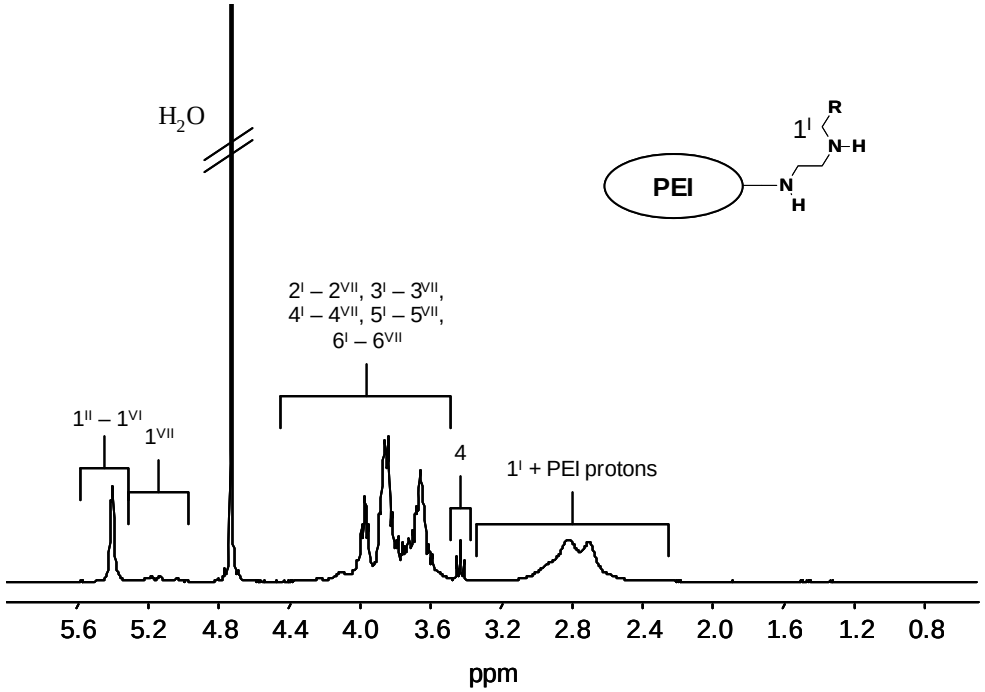

Figure 4-SI ^{1}H NMR spectrum of **6-Mal-VII** with structure **C** based on the educt ratio **PEI-II**/**Mal-VII** 1 : 0.5 (R = reductively coupled maltoheptaose unit; Scheme 1).

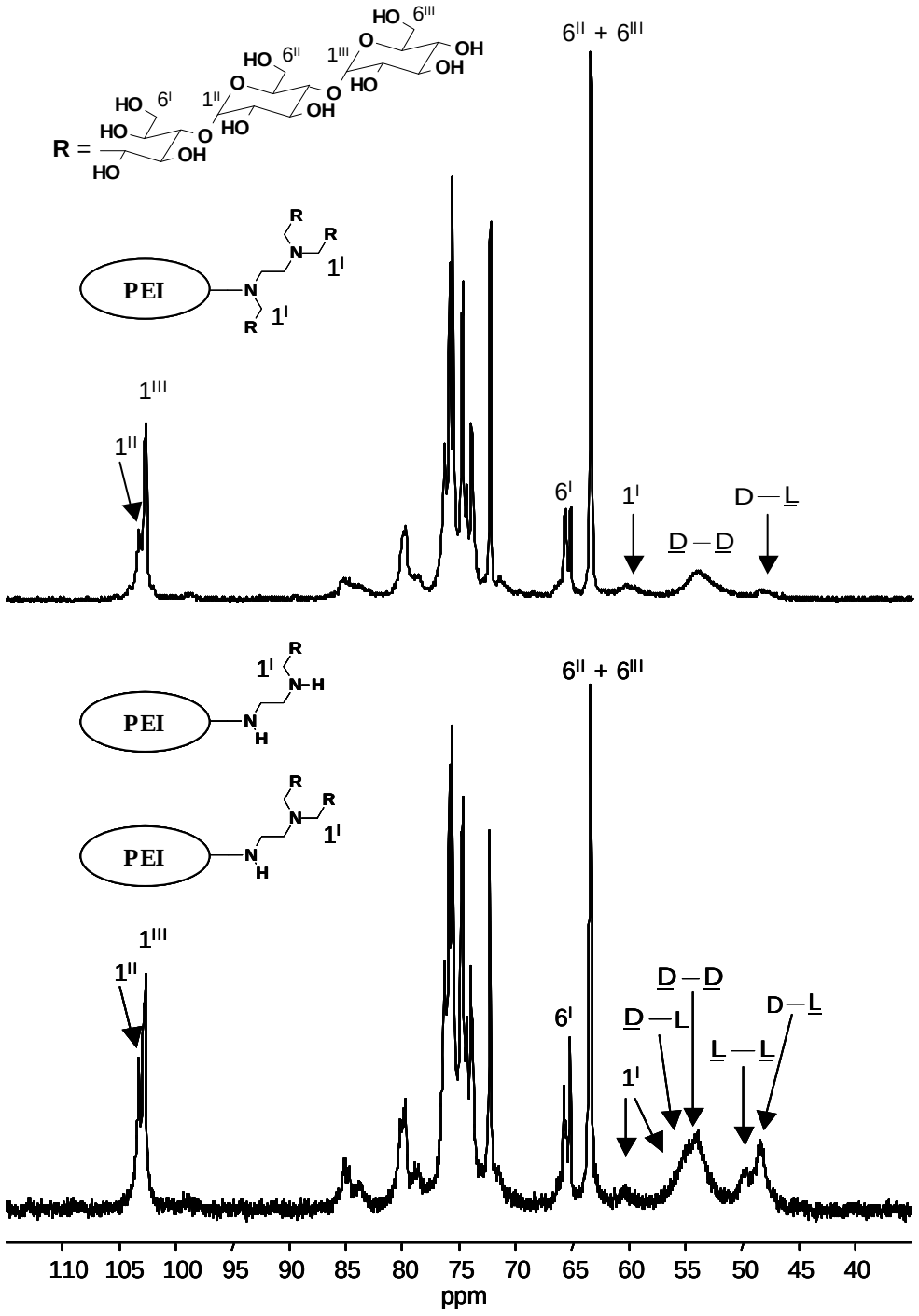

Figure 5-SI ^{13}C NMR spectra of **1-Mal-III** with structure **A** (top) based on the educt ratio **PEI-I**/**Mal-III** 1 : 5 and **3-Mal-III** with transition from structure **A** to **B** (bottom) based on the educt ratio **PEI-III**/**Mal-III** 1 : 2.

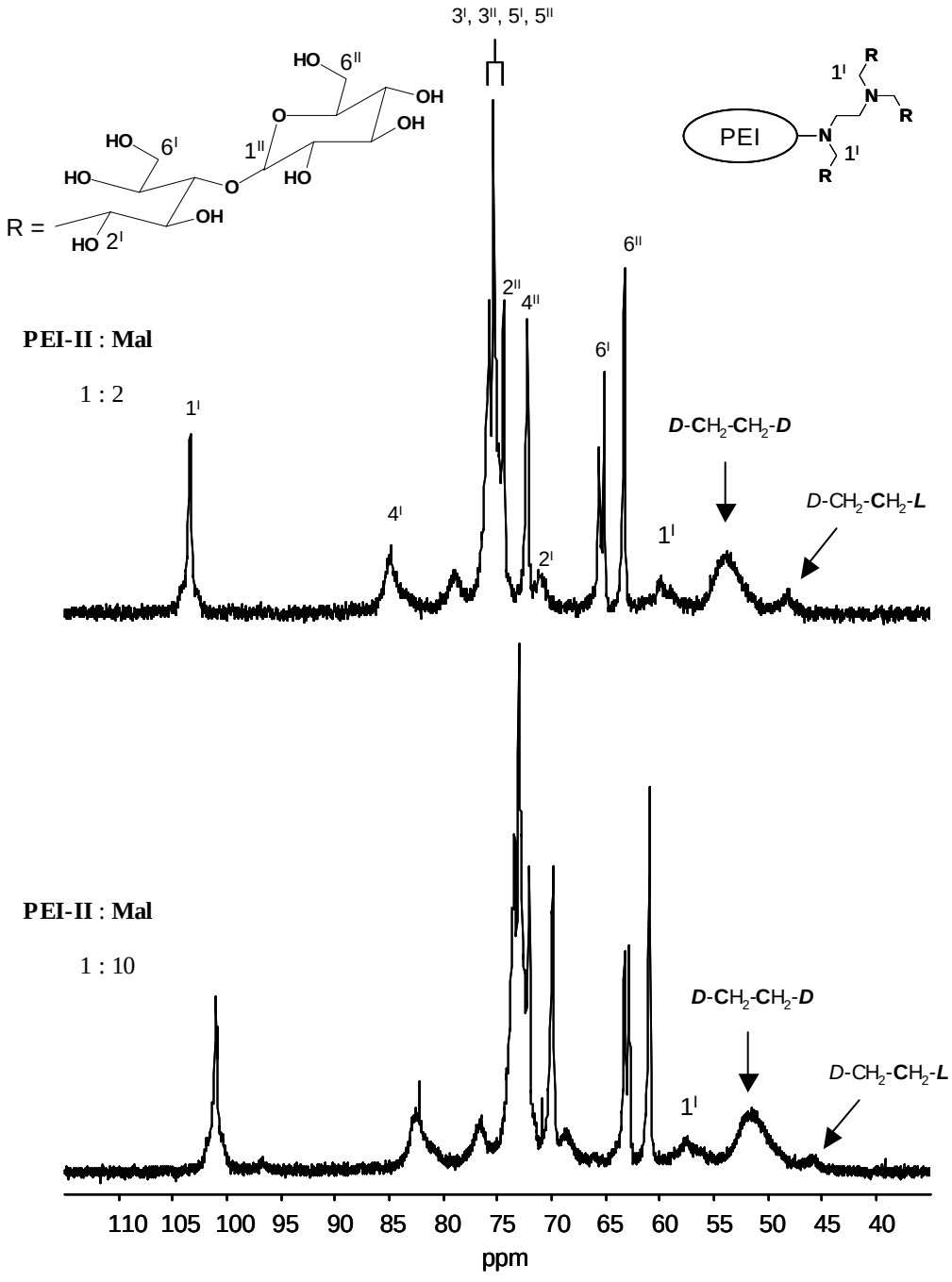

Figure 6-SI ^{13}C NMR spectra of **2-Mal** obtained from educt ratio **PEI-II**/**Mal** 1 : 2 and 1 : 10, respectively.

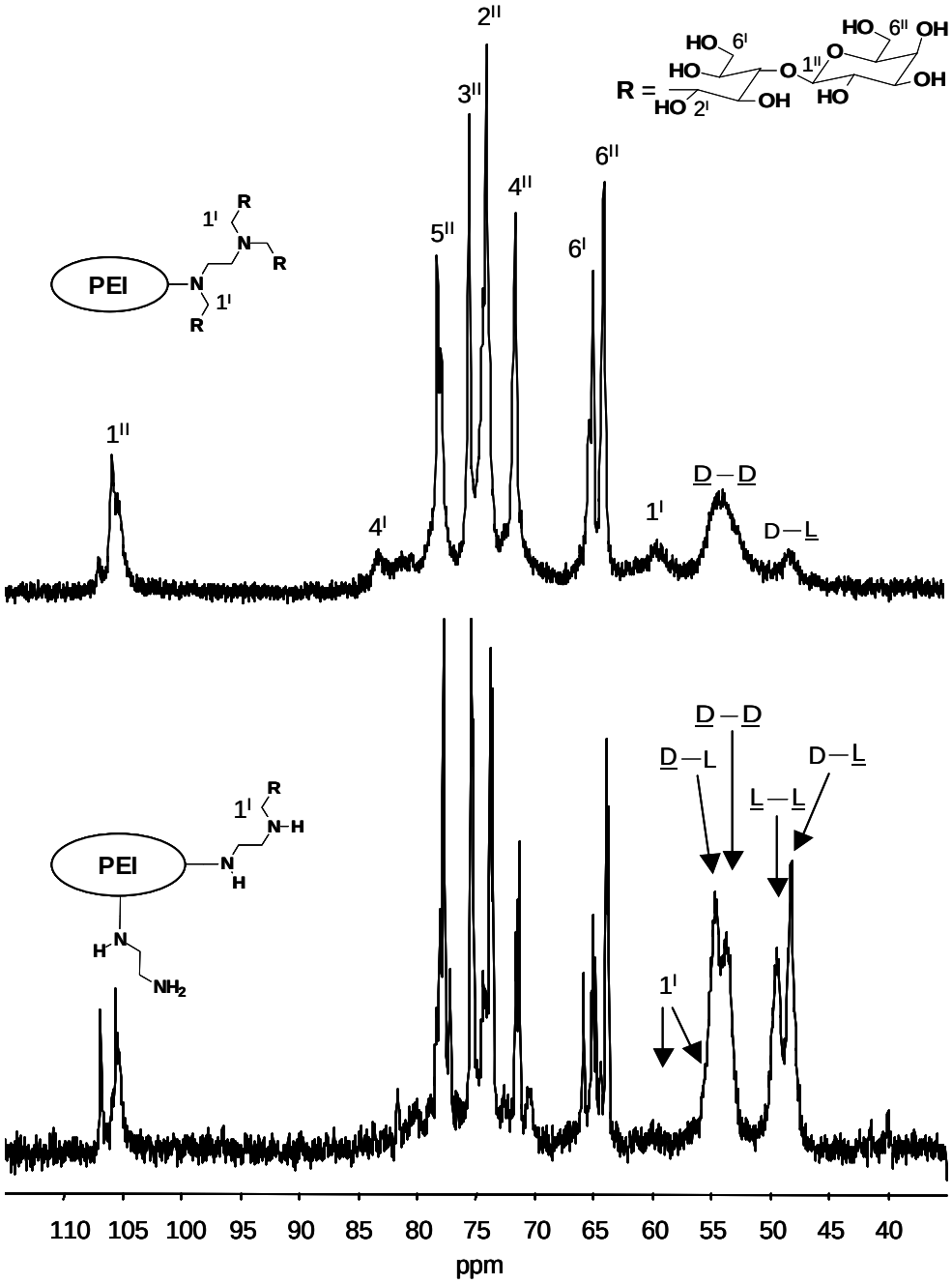

Figure 7-SI ^{13}C NMR spectrum of **2-Lac** with structure **A** (top) and **6-Lac** with structure **C** (bottom) based on the educt ratio **PEI-II**/Lac 1 : 5 and 1 : 0.4, respectively.

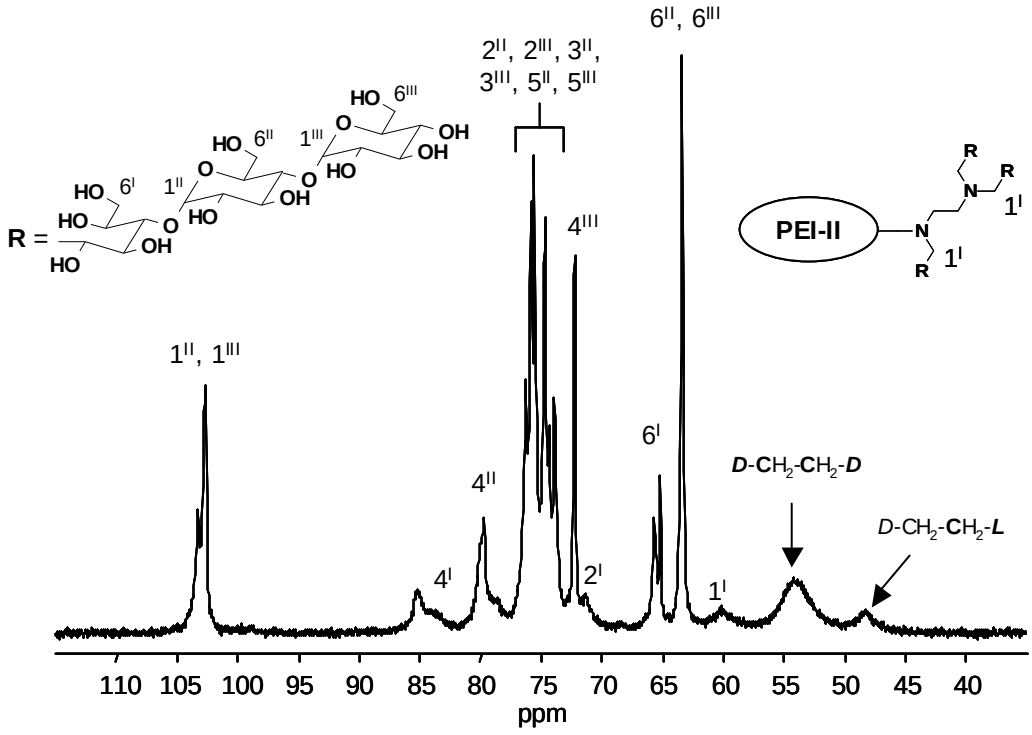

Figure 8-SI ^{13}C NMR spectra of **2-Mal-III** based on the substrate ratio **PEI-II/Mal-III** 1 : 5.

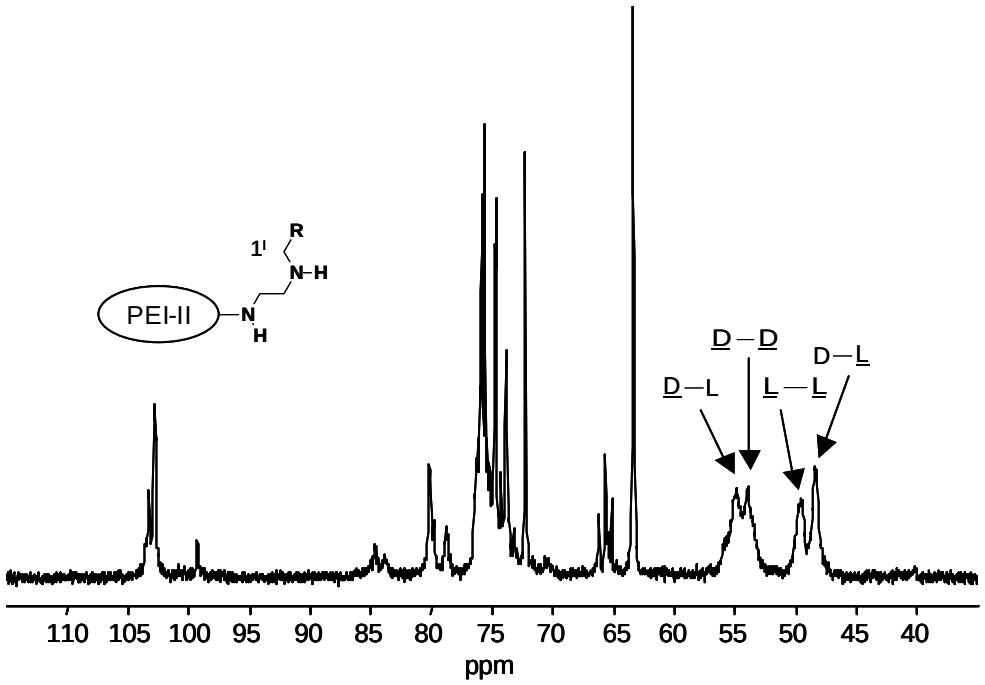

Figure 9-SI. ^{13}C NMR spectra of (A) **4-Mal-III** with structure **B** based on the substrate ratio **PEI-II**/**Mal-III** 1 : 0.5 (R = reductively coupled maltotriose).

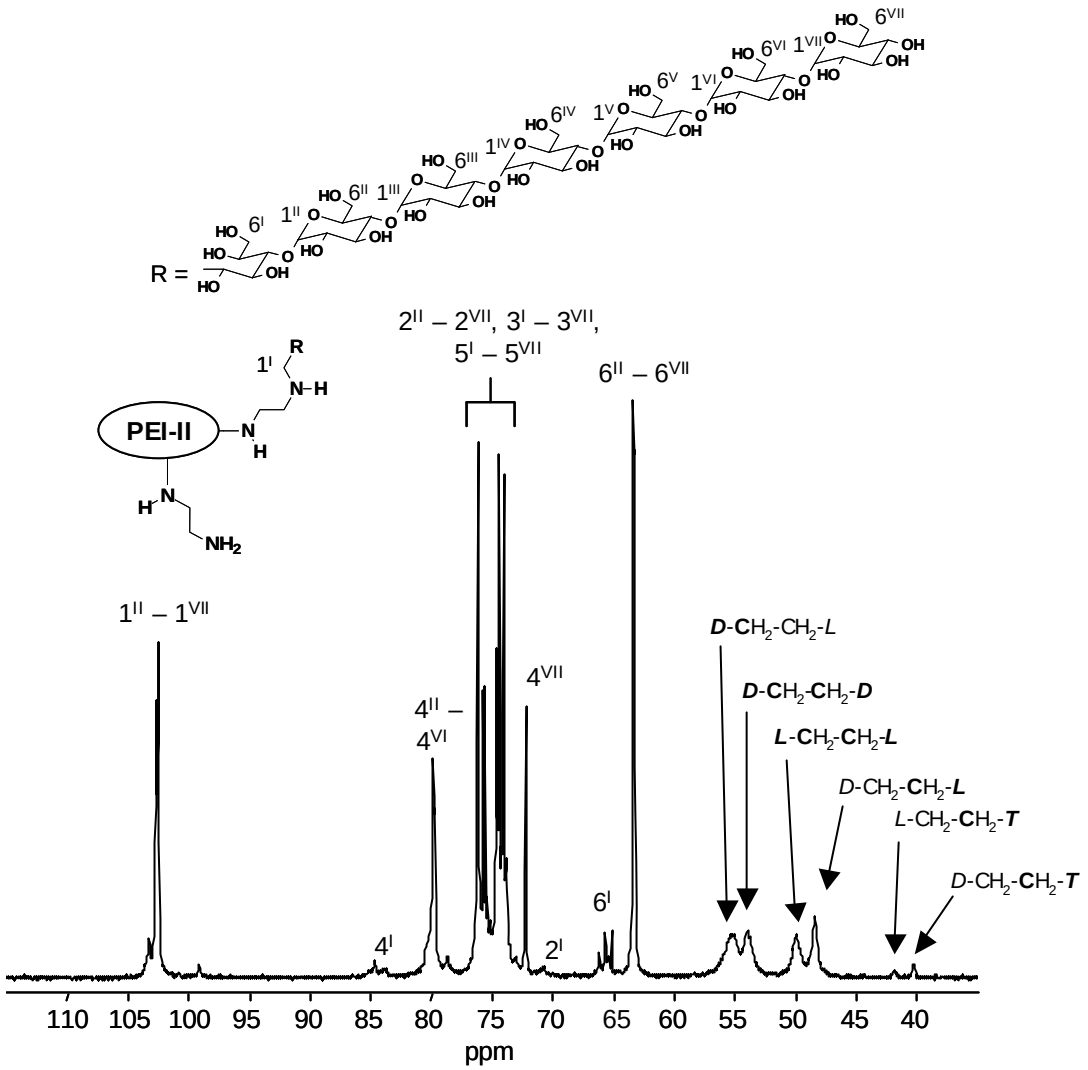

Figure 10-SI ^{13}C NMR spectrum of **6-Mal-VII** with structure **C** based on the educt ratio **PEI-II**/**Mal-VII** 1 : 0.5 (R = reductively coupled maltoheptaose unit).

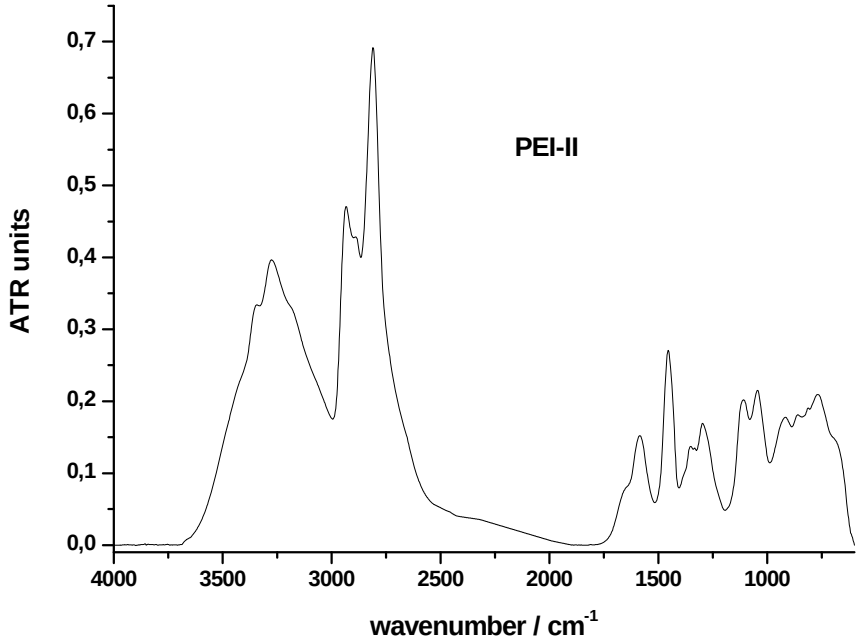

Figure 11-SI. ATR-IR spectrum of **PEI-II**.

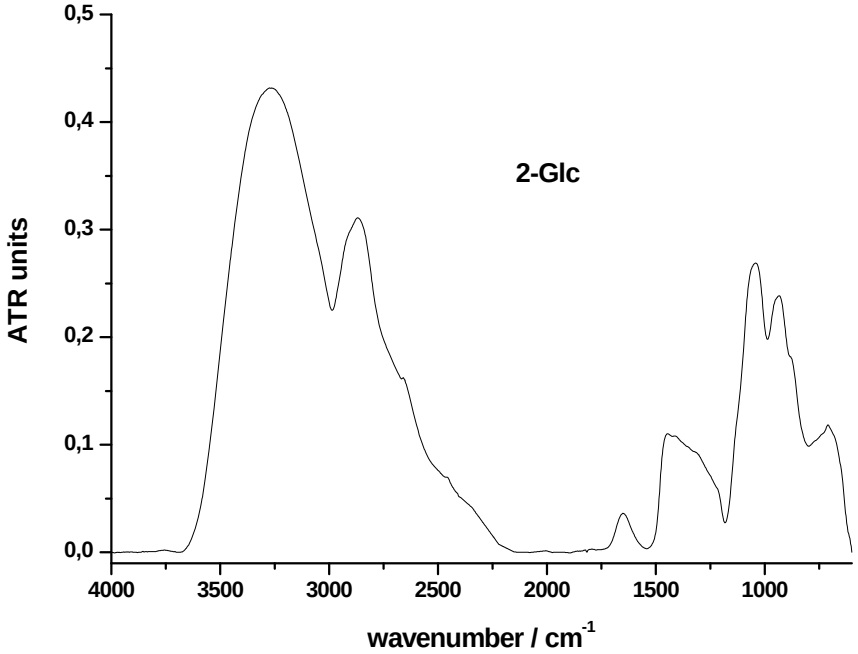

Figure 12-SI. ATR-IR spectrum of **2-Glc** with structure **A**.

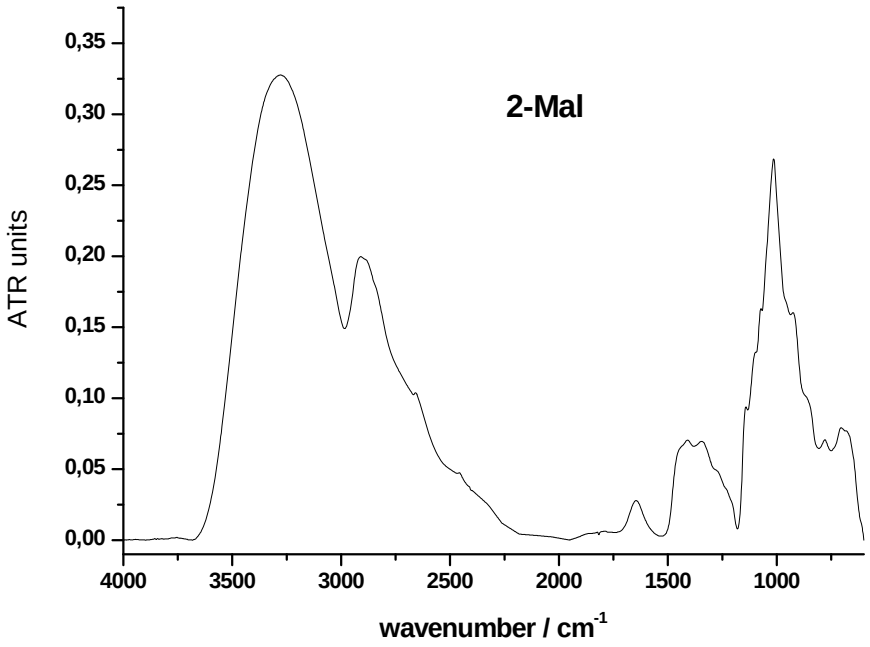

Figure 13-SI. ATR-IR spectrum of **2-Mal** with structure **A**.

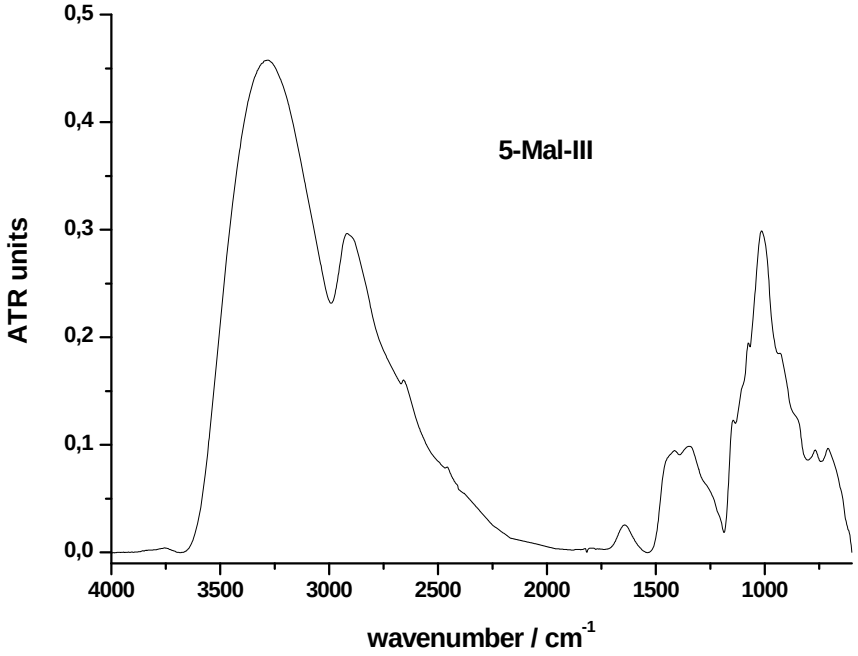

Figure 14-SI. ATR-IR spectrum of **5-Mal-III** with structure **B**.

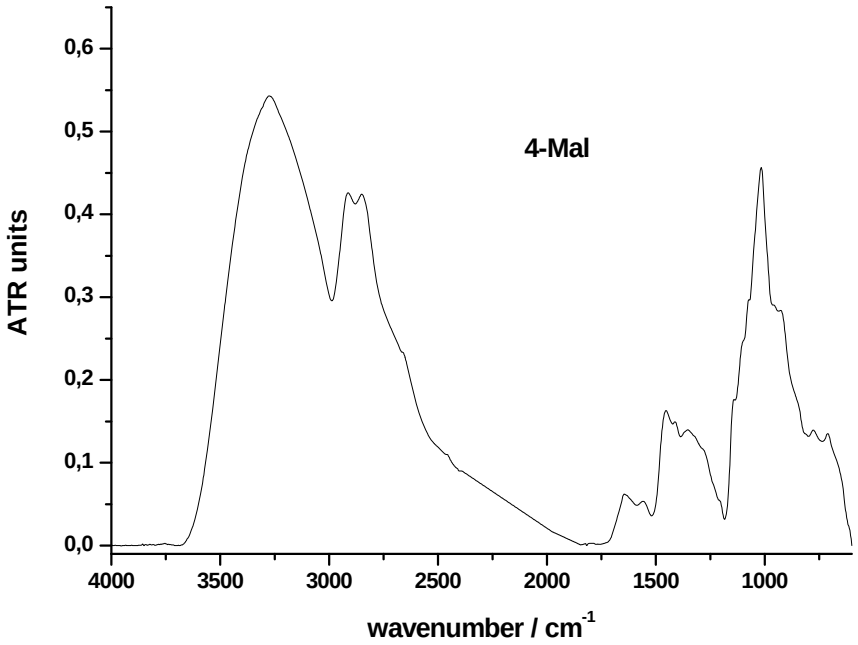

Figure 15.**-SI** ATR-IR spectrum of **4-Mal** with structure **B**.

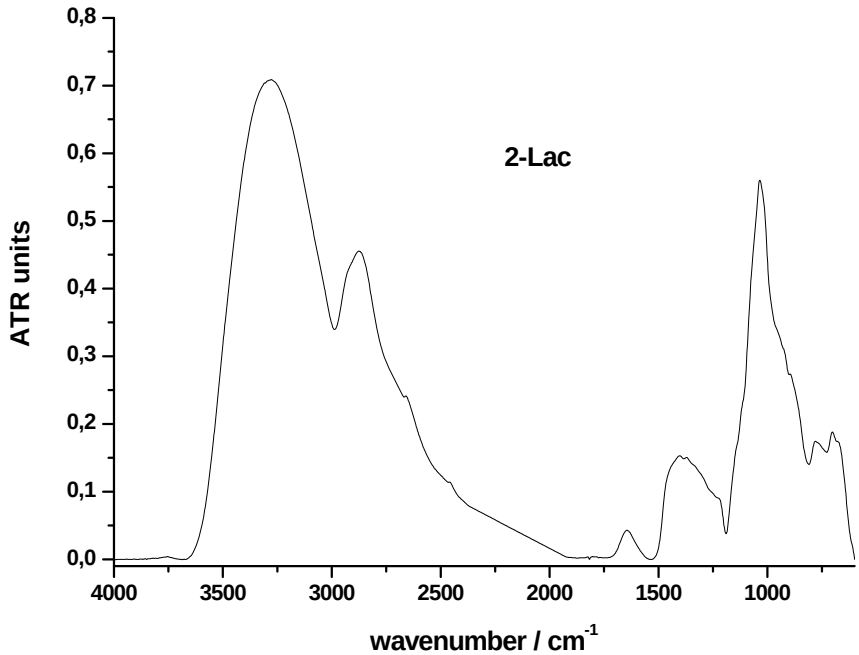

Figure 16-SI. ATR-IR spectrum of **2-Lac** with structure **A**.

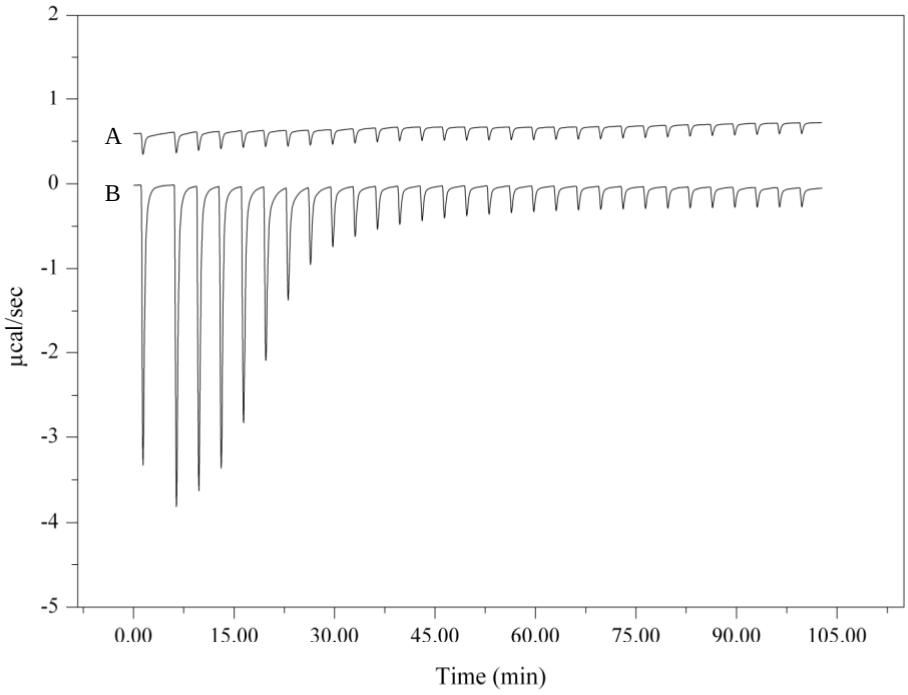

Figure 17-SI. Binding of ATP with **7-Mal-III** (A) Titration of ATP (0.1 mM) to HEPES buffer and (B) to **7-Mal-III** in HEPES buffer at 25˚C. Graphs show the calorimetric traces (heat flow against time).

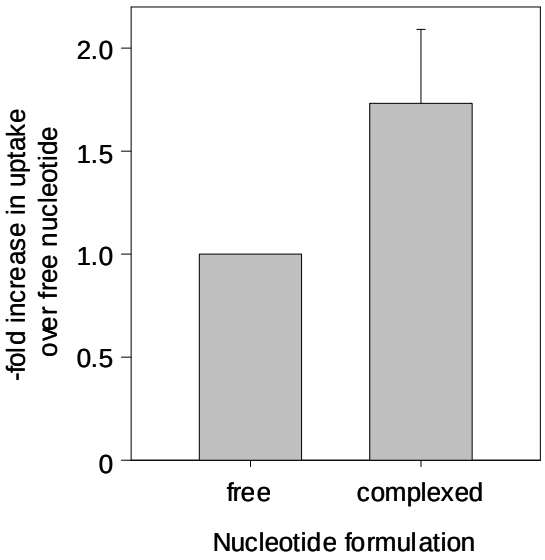

Figure 18-SI -fold increase in nucleotide uptake upon complexation (HepG2 cells): procedure as mentioned for Figure 8.

Table 1-SI. Influence of the substrate ratio **PEI-II** : oligosaccharide (OS) and **PEI-III** : OS on the degree of functionalization (DF), total degree of functionalization (TDF) of modified PEI and the determination of the degree of T, L and D units obtained from elemental analysis.

Substrate[a]	PEI	Educt ratio PEI : OS	DF for L + 2xT[b,c] %	TDF for L + 2xT + D[b,d] %	T unit[b] %	L unit[b] %	D unit[b] %
3-Mal (**A**)	**PEI-III**	1 : 4.25	91	70	-	9	91
5-Mal (**B**)	**PEI-III**	1 : 0.5	-[e]	-[e]	-[e]	-[e]	-[e]
3-Mal-III (**A**)	**PEI-III**	1 : 4.25	78	60	-	22	78
5-Mal-III (**B**)	**PEI-III**	1 : 2	48	37	-	51	49
7-Mal-III (**C**)[f]	**PEI-III**	1 : 0.4	30	21	3	67	30
					7[e]	53[e]	40.0[e]
2-Lac (**A**)	**PEI-II**	1 : 5	80	61	-	21	79
6-Lac (**C**)[g]	**PEI-II**	1 : 0.4	30	23	2	67	31
					6[e]	52[e]	42[e]
3-Lac (**A**)	**PEI-III**	1 : 4.25	50	-	-	-	-
5-Lac (**B**)	**PEI-III**	1 : 0.6	44	34	-	55	45

[a] Character in brackets presents structure for PEI derivative in Scheme 1. [b] Calculation based on elemental analysis; further details are given in Supporting Information. [c] 2xT means that two oligosaccharides can be coupled on one T unit. L means that one oligosaccharide can be coupled on the L unit. [d] All branching units are considered for the calculation of functionalization. [e] Degree of structure units determined by quantitative ^{13}C NMR. [f] degree of branching 93 %, using Fréchet equation, based on quantitative ^{13}C NMR. [g] degree of branching 94 %, using Fréchet equation, based on quantitative ^{13}C NMR.

Table 2-SI. Comparison of ^{13}C chemical shifts of T (-NH_2), L (-NHR) and D (-NR_2) units for **PEI-II** and **PEI-III** and (oligo-)saccharide-modified PEI based on modified **PEI-II** and **PEI-III** in D_2O.

Substrate		**D units**			**L units**			**T units**	
	Structure	***D*-CH_2-CH_2-*T***	**D-CH_2-CH_2-*L***	***D*-CH_2-CH_2-*D***	***L*-CH_2-CH_2-*T***	***L*-CH_2-CH_2-*L***	***D*-CH_2-CH_2-*L***	***L*-CH_2-CH_2-*T***	***D*-CH_2-CH_2-*T***
PEI-II	-	58.7	55.6, 56.7	53.5, 54.4	53.2, 53.3	50.2	48.1	42.4	40.33
2-Glc	**A**	-	-	53.3	-	-	47.8	-	-
2-Mal	**A**	-	-	53.9	-	-	48.2	-	-
4-Mal	**B**	-	54.7	53.9	-[b]	49.4	48.3	-	-
6-Mal	**C**	-[a,b]	55.1	53.7	-[b]	49.6	48.2	41.5	40.0
2-Mal-III	**A**	-	-	54.1	-	48.3	48.3	-	-
4-Mal-III	**B**	-	54.9	53.9	-[b]	50.0	48.4	-	-
6-Mal-III	**C**	-[a,b]	55.3	54.0	-[b]	49.9	48.4	41.7	40.3
6-Mal-VII	**C**	58.4	55.2	54.0	54.0	49.4	48.4	41.8	40.3
2-Lac	**A**	-	-	54.0	-	-	48.2	-	-
6-Lac	**C**	-[a,b]	54.6	53.7	-[b]	49.5	48.2	41.5	40.0
PEI-III	**-**	58.8, 58.9	55.8	53.7, 54.6	53.3, 53.7	50.4, 50.5	48.3	42.6	40.5
3-Mal	**A**	-	-	53.8	-	-	48.1	-	-
5-Mal-III	**B**	-	54.9	53.9	-[b]	49.7	48.4	-	-
7-Mal-III	**C**	-[a,b]	54.9	53.9	-[b]	49.7	48.4	41.6	40.1

[a] Not observable or not detectable compared to unmodified PEI-II. [b] Overlapped by other branching units ***D*-CH_2-CH_2-*L*** and ***D*-CH_2-CH_2-*D***.

Table 3-SI. ^{13}C signal assignment for PEI-bonded glucose (**Glc**), maltose (**Mal**) and maltotriose (**Mal-III**)[a,b]

substrate	reductive unit[c]																	
	1	2	3	4	5	6												
Glc	59.4	71.6	78.2	76.7	73.7	65.7												
	reductive unit I [c]						terminal unit II											
Mal	60.3	71.4	74.5	85.4	75.5	65.3	103.6	74.6	75.9	72.3	75.6	63.4						
	reductive unit I[c]						middle unit II						terminal unit III					
Mal-III	60.3	71.5	74.5	85.4	75.6	65.3	103.4	74.4	76.4	79.8	74.0	63.4	102.7	74.7	75.9	72.3	75.7	63.5

[a] Solvent: D_2O; reference: internal sodium salt of 3-(trimethylsilyl)propionic acid-2,2,3,3-d_4 ($\delta(^{13}C) = 0$ ppm). [b] For atom number compare Figures 3 and 5-SI. For signal groups or broadened signals the given $\delta(^{13}C)$ value is the center. [c] reductive unit is connected to the PEI scaffold by secondary or primary amino surface groups of PEI.